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Temporal net flux pattern of nutrients across splanchnic tissues in wethers consuming different forages

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Abstract

Wethers consumed alfalfa, ryegrass–wheat or bermudagrass hay three times daily (8 h feeding interval). In Experiment 1 (ad libitum intake), DE intake was 2.96, 3.29 and 2.40 Mcal day^{-1} (SE 0.316), and digestible N intake was 17.7, 10.2 and 5.5 g day^{-1} (SE 0.99) for alfalfa, ryegrass–wheat and bermudagrass, respectively. Splanchnic oxygen consumption over an 8 h period was affected ($P = 0.05$) by a treatment by time post-feeding interaction because of a similar interaction ($P = 0.08$) in hepatic oxygen consumption. Alpha-amino N release by the portal-drained viscera was greater for alfalfa versus grasses and differed ($P < 0.01$) among times. Ammonia N release by the portal-drained viscera was affected by a treatment by time interaction. In Experiment 2 (2% of BW DM intake), portal-drained viscera oxygen consumption did not differ ($P > 0.10$) with time post-feeding, and portal-drained viscera ammonia N release exhibited ($P = 0.07$) a treatment by time interaction. In conclusion, alfalfa consumed ad libitum resulted in a different temporal pattern of splanchnic bed oxygen consumption than ryegrass–wheat or bermudagrass, but it did not appear that high oxygen consumption early after feeding alfalfa impacted subsequent oxygen use. Samples should be taken throughout an 8 h feeding interval, particularly early after feeding, to assess average net flux of nutrients across splanchnic tissues over the interval with ad libitum consumption or with a moderately restricted level of feed intake. © 1997 Elsevier Science B.V.

Keywords: Sheep; Metabolism; Forage; N net flux; Splanchnic oxygen consumption

1. Introduction

Splanchnic tissues are metabolically active, accounting for a substantial proportion of whole body heat production (Ferrell, 1988). Physiological workload, as depicted by DE or ME intake, influences energy consumption by splanchnic tissues (Johnson et al., 1990). Other factors such as chemical or physical characteristics of diets can have impact as well (Rǫmpala et al., 1988, 1990). Reynolds et al. (1991) suggested that dietary characteristics may affect splanchnic tissue heat energy production relative to ME intake via simple dilution of maintenance energy costs. This implies that splanchnic tissues maintain excess metabolic machinery for a particular quantity and perhaps array of nutrients entering the

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gut and liver. Perhaps this occurs in order to minimize excessive quantities of metabolites such as ammonia reaching peripheral blood, which can deleteriously impact peripheral tissue integrity (Visek, 1984). Because forages differ in rate and extent of ruminal degradation, it is possible that the degree of excess metabolic machinery maintained by splanchnic tissues varies among forage sources, thereby influencing the quantity of energy and proportion of that absorbed available to peripheral tissues. Therefore, this study was conducted to determine net fluxes of oxygen and nitrogenous compounds across the PDV and liver in wethers consuming legume, temperate grass or tropical grass at different times in an 8 h feeding interval.

2. Materials and methods

2.1. Experiment 1: Animals

Crossbred (Suffolk × Rambouillet–Dorset) wethers ($n = 9$; 37 ± 1.8 kg BW; 11 months old) were surgically fitted with chronic indwelling catheters in a hepatic vein, the portal vein and a mesenteric vein and artery (Ferrell et al., 1992). Catheters were filled with heparinized (100 units mL^{-1}) saline (8.5 g L^{-1}) solution at surgery. The experiment began approximately 14 wk after surgery. Sheep had been used previously in other experiments with moderate-quality grass-based diets. Sheep were individually maintained in 1.1 × 1.5 m elevated pens with an expanded metal floor and had free access to water. Sheep were cared for in accordance with guidelines of Consortium (1988).

2.2. Experiment 1: Diets

Sheep consumed ad libitum (offered at 105 to 110% of consumption on the preceding few days) coarsely chopped alfalfa ((A) *Medicago sativa*; post-anthesis), ryegrass (*Lolium multiflorum*; post-anthesis)-wheat ((RW) *Triticum aestivum*; post-anthesis) or bermudagrass ((B) *Cynodon dactylon*; vegetative growth stage) hay. Equal-sized meals were at 14:00, 22:00 and 06:00 h. At 14:00 h, sheep received 3.5 g day^{-1} of a mineral mixture containing 20% trace minerals (12% Zn, 10% Mn, 5% K, 2.5% Mg, 1.5% Cu, 0.3% I, 0.1% Co and 0.02% Se) and 80% NaCl.

2.3. Experiment 1: Sampling and analyses

The experiment was 21 days in length. Feed was sampled daily on day 12 through 21 to form a composite. Feces was collected in canvas bags on day 15 through 18, and a composite sample was formed from 10% aliquots of daily excretion. Percentages of time in a 24 h period spent standing versus lying, and eating, ruminating and idle were determined by observations at 15 min intervals on day 14.

Metabolism crates were used to house sheep during blood collections, with three sheep sampled daily (one/treatment) on day 18, 19, 20 and 21. A priming dose (15 mL) of para-aminohippuric acid (2.25%; wt/vol) was administered into the mesenteric vein catheter at 30 min before the collection of samples, followed by continuous infusion (0.8 mL min^{-1}). Body weight was determined immediately after the last sample was obtained.

Blood was withdrawn from portal and hepatic venous and arterial catheters starting at 07:40 h at 20 min intervals for an 8 h period (24 samples) to represent an 8 h feeding interval, with 5 min intervals between sheep. The treatment order for sampling differed among days. A 1 mL sample was obtained anaerobically into a heparinized syringe and placed in ice. Oxygen saturation and hemoglobin concentration (OSM 2; Radiometer Corporation, Copenhagen, Denmark) were measured immediately, and values for three samples of an hour were averaged for calculation of oxygen concentration as described by Eisemann and Nienaber (1990). At three consecutive sampling times, 3 mL of blood were placed in a tube containing potassium oxalate and sodium fluoride and situated in ice to form hourly composites (9 mL). On the day of sampling, a 1.5-mL aliquot of the hourly composite samples was diluted with deionized water (4.5 mL) and subjected to automated procedures for para-aminohippuric acid, alpha-amino N (AAN), urea N (UN) and ammonia N (AMN), as described by Eisemann and Nienaber (1990). Net metabolite fluxes were calculated based on venoarterial concentration differences and whole blood flows (Burrin et al., 1991). Net fluxes were

calculated based on para-aminohippuric acid and metabolite concentrations at each time (method 1) and also based on concentrations averaged over time (method 2).

Fecal composites were dried at 55°C and allowed to air-equilibrate. Hay and fecal samples were ground to pass a 1 mm screen and analyzed for DM, ash, Kjeldahl N, energy (AOAC, 1984) and NDF (Goering and Van Soest, 1970; without sodium sulphite, decalin or ethoxyethanol). Hay samples also were analyzed for ADF and ADL (Goering and Van Soest, 1970). Cellulose was estimated as loss in weight upon H_2SO_4 treatment and hemicellulose as the difference between NDF and ADF. The average of feed intake on day 12 through the day of blood sampling was used to calculate digestibilities.

Data were analyzed by the General Linear Models procedure of SAS (1990), with a model consisting of forage treatment, animal within forage treatment (error for forage treatment), time within the feeding interval and the interaction between forage treatment and time. The interaction was considered significant with $P \leq 0.10$. With a nonsignificant interaction, orthogonal contrasts were used to test effects of forage source (A versus RW and B) and grass source (RW versus B). All data from one wether were omitted from analyses because of a non-patent arterial catheter (A treatment), and the portal catheter of one wether (RW) and hepatic catheter of another (B) were non-patent. Therefore, the number of observations for main effect treatments was eight for feed intake, digestibility and behavior, seven for portal-drained viscera (PDV) net fluxes (A: 2; RW: 2; B: 3), seven for splanchnic bed net fluxes (A: 2; RW: 3; B: 2) and six for hepatic net fluxes (two per treatment). Linear, quadratic and cubic effects of time post-feeding were tested for some variables with the General Linear Models procedure of SAS (1990). Prior to behavior data analysis, effects of 8 h feeding interval and the feeding interval–treatment interaction were tested with a split-plot design. In addition, effects of method of determination were tested as a split-plot, with a model containing treatment, animal within treatment, method and the interaction between treatment and method.

2.4. Experiment 2

The eight wethers with functional arterial catheters in Experiment 1 (34.0 ± 2.0 kg BW) were used in a subsequent experiment with a crossover design. Wethers consumed 2.0% BW (DM) of A or B. Other methodology was that described for Experiment 1. Data were analyzed as a split-plot, with forage treatment, period, animal, animal within period (error for treatment effect), time and the treatment by time interaction in the model. A number of catheters became non-patent in the second period or were so in both. Thus, only data for four animals in each period (two per treatment) were analyzed for intake, digestibility and PDV net fluxes.

3. Results

3.1. Experiment 1

Alfalfa differed from grasses in chemical composition as expected, although concentrations of NDF and ADL did not greatly differ between RW and B

Table 1
Composition (% of DM) of alfalfa, ryegrass-wheat and bermudagrass hay consumed by wethers.

Item	Experiment 1			Experiment 2	
	Alfalfa	Ryegrass-wheat	Bermuda grass	Alfalfa	Bermuda grass
Ash (%)	8.7	6.9	6.1	8.8	6.8
GE (Mcal kg^{-1})	4.05	4.16	4.13	4.07	4.14
CP (%)	14.7	9.4	6.4	15.1	7.1
NDF (%)	57.4	70.4	76.9	57.8	76.1
ADF (%)	40.2	33.3	34.9	40.2	34.9
ADL (%)	6.7	4.2	5.1	6.8	5.0
Cellulose (%)	33.5	29.1	29.8	33.4	29.7
Hemicellulose (%)	17.2	37.1	42.0	17.6	41.2

(Table 1). Dry matter and OM intakes were not affected ($P > 0.10$) by treatment (Table 2). Digestibilities of OM and NDF were greater for RW versus B ($P < 0.01$), and N digestibility was greater ($P < 0.01$) for A than for grasses and less ($P = 0.09$) for B versus RW. Digestible OM and DE intakes were similar ($P > 0.10$) between A and grasses but greater ($P = 0.09$) for RW than for B.

Behavior measures did not significantly differ among 8 h feeding intervals, and interactions between 8 h feeding interval and forage treatment were nonsignificant ($P > 0.10$). Percentages of time spent standing (A: 97, 28, 28, 11, 11, 19, 22 and 11%; RW: 100, 58, 11, 14, 25, 31, 28 and 13%; B: 100, 22, 11, 17, 19, 17, 19 and 11% for 1, 2, 3, 4, 5, 6, 7 and 8 h post-feeding, respectively (SE 6.3)) and eating were affected by interactions ($P = 0.10$ and 0.04, respectively) between treatment and time post-

Table 2
Feed intake, digestion, behavior and splanchnic tissue blood flow and net flux of nutrients in wethers consuming different forages *ad libitum* (Experiment 1) [a]

Item	Treatment			SE	Effect [b]
	Alfalfa	Ryegrass-wheat	Bermuda grass		
DM intake (g day^{-1})	1024	1080	930	96.7	NS
OM					
Intake (g day^{-1})	955	1033	899	92.6	NS
Digestion:					
Per cent (%)	69.3	71.2	59.7	1.69	G
g day^{-1}	661	734	537	70.7	g
NDF					
Intake (g day^{-1})	600	781	736	70.2	NS
Digestion:					
Per cent (%)	68.6	72.5	58.8	1.75	G
g day^{-1}	412	567	433	54.4	NS
N					
Intake (g day^{-1})	24.6	16.7	9.9	1.50	F,G
Digestion:					
Per cent (%)	71.7	61.0	56.2	1.78	F,g
g day^{-1}	17.7	10.2	5.5	0.99	F,G
DE intake (Mcal day^{-1})	2.96	3.29	2.40	0.316	g
Behavior (% of time)					
Ruminating	22.6	41.7	41.7	1.94	F
Idle	60.1	31.6	36.8	1.77	F,g
Whole blood flow (L h^{-1})					
Portal vein	117	122	123	17.6	NS
Hepatic vein	154	154	153	19.4	NS
Portal-drained viscera					
oxygen consumption (mmol h^{-1})	137	159	141	29.6	NS
Alpha-amino N net flux (mmol h^{-1})					
Portal-drained viscera	26.1	13.7	9.3	4.11	F
Hepatic	-19.3	-11.5	-22.7	4.17	NS
Splanchnic	6.8	0.5	-14.1	4.80	f,g
Urea N net flux (mmol h^{-1})					
Portal-drained viscera	-25.6	-20.7	-16.9	7.32	NS
Hepatic	68.4	36.5	33.5	7.56	F
Splanchnic	42.8	17.4	15.4	8.29	f
Ammonia N net flux (mmol h^{-1})					
Hepatic	-21.6	-8.3	-10.2	3.03	F
Splanchnic	-2.0	-0.5	-2.3	1.59	NS

[a] Averages of blood flows and net fluxes at each hour of an 8-h feeding interval.[b] F and f = alfalfa *vs* ryegrass-wheat and bermudagrass ($P < 0.05$ and 0.10, respectively); G and g = RW *vs* B ($P < 0.05$ and 0.10, respectively); NS = not significant ($P > 0.10$).

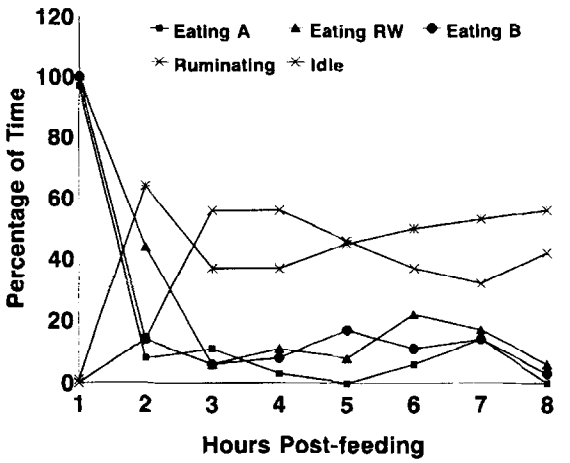

Fig. 1. Percentages of time eating, ruminating and idle at different times post-feeding in wethers consuming ad libitum alfalfa (A), ryegrass–wheat (RW) or bermudagrass (B) hay. Pooled SE were 5.2, 4.0 and 4.8% for eating, ruminating and idle, respectively (Experiment 1).

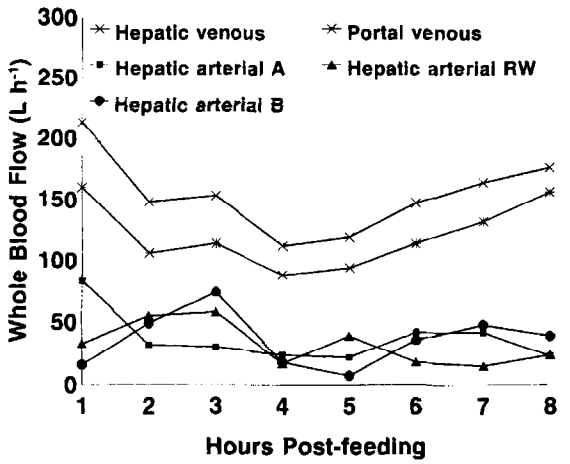

Fig. 2. Whole blood flow at different times post-feeding (based on samples collected at listed times, and at 20 min before and after) in wethers consuming ad libitum alfalfa (A), ryegrass–wheat (RW) or bermudagrass (B) hay. Pooled SE were 14.4, 9.1 and 13.2 L h^{-1} for hepatic venous, portal venous and hepatic arterial flows, respectively (Experiment 1).

feeding (Fig. 1), largely because of longer periods of time following meals spent standing and ingesting for RW versus A and B. Percentage of time ruminating varied ($P < 0.01$) with forage treatment (Table 2) and time (Fig. 1), being less for A than for grasses. Likewise, idle time differed among times ($P < 0.01$) and was greater ($P = 0.08$) for A versus grasses (Table 2 and Fig. 1).

Blood flows were similar ($P > 0.10$) among treatments (Table 2), although time affected portal and hepatic flows ($P < 0.01$). A forage treatment by time interaction existed ($P = 0.06$) in hepatic arterial blood flow (Fig. 2), with flows decreasing then increasing as time post-feeding increased (linear and quadratic; $P < 0.01$). Portal-drained viscera oxygen consumption was similar among treatments and times post-feeding ($P > 0.01$; Table 2). Oxygen consumption by the liver ($P = 0.08$) and splanchnic bed ($P = 0.05$) was affected by interactions between forage treatment and time post-feeding, with a greater difference among treatments at 1 h post-feeding versus other times (Fig. 3).

Portal-drained viscera release of AAN was greater ($P < 0.01$) for A than for RW and B (Table 2) and differed among times post-feeding similarly regardless of forage treatment (Fig. 4). Hepatic AAN uptake was not altered by treatment ($P > 0.10$); splanchnic AAN net flux was greater ($P = 0.09$) for A versus grasses and for RW versus B ($P = 0.09$). Neither hepatic uptake nor splanchnic net flux of AAN varied with time post-feeding ($P > 0.10$). Urea N net fluxes were not affected by time post-feeding.

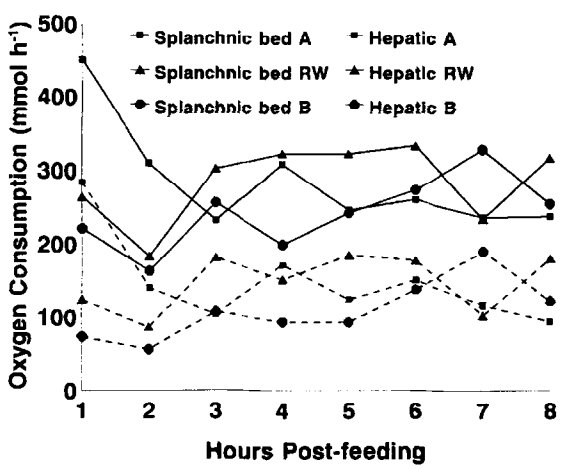

Fig. 3. Splanchnic bed and hepatic oxygen consumption at different times post-feeding (based on samples collected at listed times, and at 20 min before and after) in wethers consuming ad libitum alfalfa (A), ryegrass–wheat (RW) or bermudagrass (B) hay. Pooled SE were 45.4 and 38.7 mmol h^{-1} for splanchnic bed and hepatic consumption, respectively (Experiment 1).

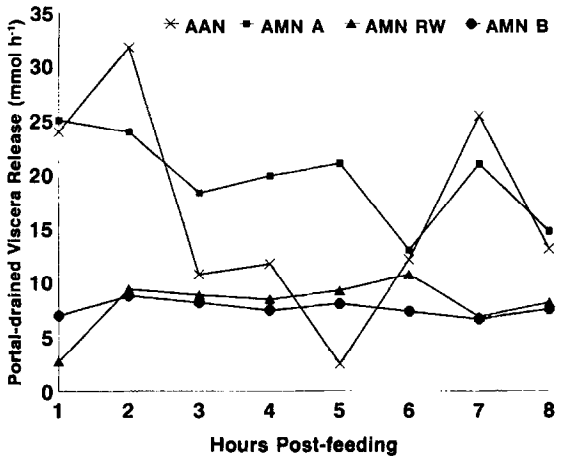

Fig. 4. Portal-drained viscera release of alpha-amino N (AAN) and ammonia N (AMN) at different times post-feeding (based on samples collected at listed times, and at 20 min before and after) in wethers consuming ad libitum alfalfa (A), ryegrass–wheat (RW) or bermudagrass (B) hay. Pooled SE were 5.11 and 2.22 mmol h^{-1} for AAN and AMN, respectively (Experiment 1).

Uptake of UN by the PDV was not affected by treatment ($P > 0.10$), although hepatic ($P = 0.04$) and splanchnic releases ($P = 0.07$) were greater for A versus grasses. Ammonia N release by the PDV was affected by a forage treatment by time post-feeding interaction ($P = 0.10$; Fig. 4), which appeared largely owing to greater release for A versus grasses

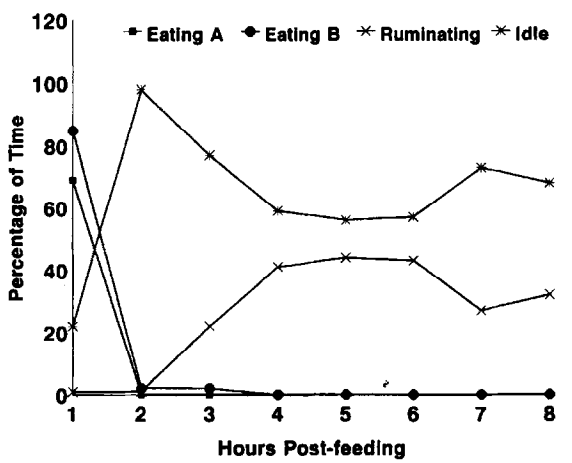

Fig. 5. Percentages of time eating, ruminating and idle at different times post-feeding in wethers consuming 2.0% of BW (DM) of alfalfa (A) or bermudagrass (B) hay. Pooled SE were 2.5, 5.1 and 5.3% for eating, ruminating and idle, respectively (Experiment 2).

Table 3
Feed intake, digestion, behavior, portal venous blood flow and portal-drained viscera (PDV) net flux of nutrients in wethers consuming different forages at 2.0% of BW (DM; Experiment 2) [a]

Item	Treatment		SE
	Alfalfa	Bermuda-grass	
DM intake (g day^{-1})	678	678	
OM			
Intake (g day^{-1})	619	632	0.69
Digestion:			
Per cent (%)	65.9 [c]	60.5 [b]	0.37
g day^{-1}	411 [c]	384 [b]	3.20
NDF			
Intake (g day^{-1})	392 [b]	516 [c]	6.28
Digestion:			
Per cent (%)	65.5 [c]	59.6 [b]	0.27
g day^{-1}	258 [b]	309 [c]	2.12
N			
Intake (g day^{-1})	16.4 [c]	7.7 [b]	0.44
Digestion:			
Per cent (%)	68.5 [c]	57.0 [b]	0.43
g day^{-1}	11.3 [c]	4.4 [b]	0.44
DE intake (Mcal day^{-1})	1.84 [c]	1.72 [b]	0.014
Behavior (% of time)			
Standing	24.5	24.5	0.94
Ruminating	21.4 [b]	31.3 [c]	1.16
Idle	70.1 [c]	57.6 [b]	1.16
Portal venous whole blood flow (L h^{-1})	122	162	32.6
PDV oxygen consumption (mmol h^{-1})	120	171	33.5
PDV alpha-amino N net flux (mmol h^{-1})	30.0 [c]	10.2 [b]	2.18
PDV urea N net flux (mmol h^{-1})	-15.1	-18.1	4.58

[a] Averages of blood flows and net fluxes at each hour of an 8-h feeding interval.[b,c] Means in a row without a common superscript differ ($P < 0.05$).

early and late in the feeding interval. Likewise, hepatic AMN uptake was greater ($P = 0.04$) for A than for RW and B, and splanchnic net flux of AAN was similar among treatments ($P > 0.10$).

3.2. Experiment 2

Digestibilities were greater ($P < 0.01$) for A than for B (Table 3), with values similar between experiments. Behavior measures did not significantly differ among 8 h feeding intervals, and interactions between 8 h feeding interval and forage treatment were nonsignificant ($P > 0.10$). The interaction between

forage treatment and time post-feeding was significant ($P = 0.02$) for eating time (Fig. 5), primarily as a result of less time eating in the first hour after feeding A versus B. Standing time, however, varied with time post-feeding similarly regardless of treatment (91, 7, 13, 10, 20, 12, 16 and 28% for 1, 2, 3, 4, 5, 6, 7 and 8 h post-feeding, respectively; SE 3.7). Rumination time was greater ($P = 0.03$) and idle time lower ($P = 0.02$) for B versus A.

Portal venous whole blood flow and PDV oxygen consumption were similar ($P > 0.10$) between A and B (Table 3). Portal venous whole blood flow varied with time post-feeding, declining from 3 to 5 h post-feeding then steadily increasing with advancing time (linear, $P = 0.08$ and quadratic, $P = 0.09$; Fig. 6). Portal-drained viscera AAN release was greater ($P = 0.02$) for A versus B, and UN uptake by the PDV was similar between treatments. Neither PDV release of AAN nor uptake of UN was affected by time post-feeding ($P > 0.10$). Ammonia N release by the PDV was affected by an interaction between forage treatment and time post-feeding ($P = 0.07$; Fig. 7). The greatest difference between forage treatments was in greater PDV AMN release for A versus B at 1 and 2 h post-feeding and lower release for A at h 5 through 8.

Table 4
Effect of method of determination on whole blood flow and net flux of nutrients across splanchnic tissues in wethers consuming different forages.

Item	Method 1 [a]			Method 2 [a]			SE	Effect [b]
	A [c]	RW [c]	B [c]	A	RW	B		
Experiment 1 [d]								
Whole blood flow (L h^{-1}):								
Portal vein	117	122	123	108	114	117	2.0	M
Hepatic vein	154	154	153	141	144	146	0.9	M,i
Hepatic arterial	37	32	36	33	30	32	1.7	
Oxygen consumption (mmol h^{-1}):								
Portal-drained viscera	137	159	141	133	152	138	1.1	M
Hepatic	149	149	110	141	144	106	1.4	M
Splanchnic	286	285	243	273	278	237	3.1	
Alpha-amino N net flux (mmol h^{-1}):								
Portal-drained viscera	26.1	13.7	9.2	24.7	13.6	9.1	0.35	
Hepatic	-19.3	-11.5	-22.6	-18.4	-11.4	-21.7	0.19	M
Splanchnic	6.8	0.5	-14.1	6.3	0.4	-13.4	0.39	M
Urea N net flux (mmol h^{-1}):								
Portal-drained viscera	-25.6	-20.7	-16.8	-23.5	-19.4	-17.0	1.29	
Hepatic	63.4	36.5	33.5	67.3	35.9	33.9	2.06	
Splanchnic	42.8	17.4	15.4	43.8	18.6	15.1	2.09	
Ammonia N net flux (mmol h^{-1}):								
Portal-drained viscera	19.6	8.0	7.5	19.1	8.1	7.6	0.13	
Hepatic	-21.6	-8.3	-10.7	-20.9	-8.8	-10.2	0.16	i
Splanchnic	-2.0	-0.5	-2.3	-1.8	-0.9	-2.4	0.11	
Experiment 2								
Portal venous whole blood flow (L h^{-1})	122		162	108		156	2.9	M
Portal-drained viscera oxygen consumption (mmol h^{-1})	120		171	109		166	3.0	M
PDV net flux (mmol h^{-1}):								
Alpha-amino N	30.0		10.2	27.5		10.6	0.99	
Urea N	-15.1		-18.1	-14.8		-17.2	0.89	
Ammonia N	12.4		12.1	10.7		11.5	0.41	M

[a] Method 1: average of flows or net fluxes each hour of an 8-h feeding interval; Method 2: based on concentrations averaged across time.[b] M = difference between methods ($P < 0.05$); i = interaction between forage treatment and method ($P < 0.10$).[c] A = alfalfa; RW = ryegrass-wheat; B = bermudagrass.[d] Experiment 1: *ad libitum* consumption; Experiment 2: 2.0% of BW DM intake.

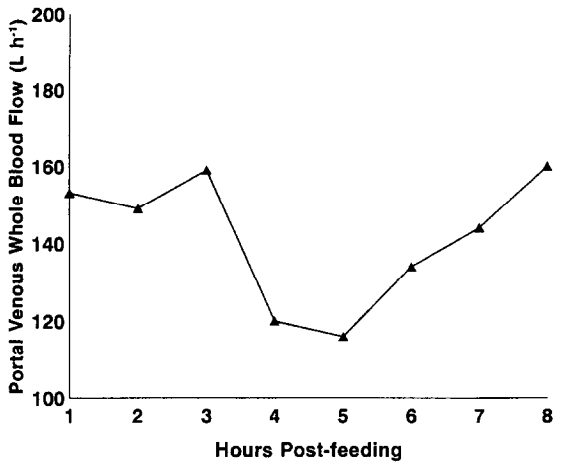

Fig. 6. Portal venous whole blood flow at different times post-feeding (based on samples collected at listed times, and at 20 min before and after) in wethers consuming 2.0% of BW (DM) of alfalfa or bermudagrass hay. The pooled SE was 12.5 L h^{-1} (Experiment 2).

3.3. Method of determination

In Experiment 1, method of determination affected portal venous blood flow ($P = 0.01$), hepatic venous blood flow ($P < 0.01$), PDV oxygen consumption ($P < 0.01$), hepatic oxygen consumption ($P = 0.01$), hepatic AAN uptake ($P = 0.03$) and splanchnic AAN net flux ($P = 0.03$), and interactions between forage treatment and method were noted for hepatic venous blood flow ($P = 0.08$) and hepatic AMN uptake ($P = 0.09$; Table 4). In Experiment 2, method altered portal venous blood flow ($P = 0.02$), PDV oxygen consumption ($P = 0.04$) and PDV AMN release ($P = 0.03$), although there were no interactions ($P > 0.10$).

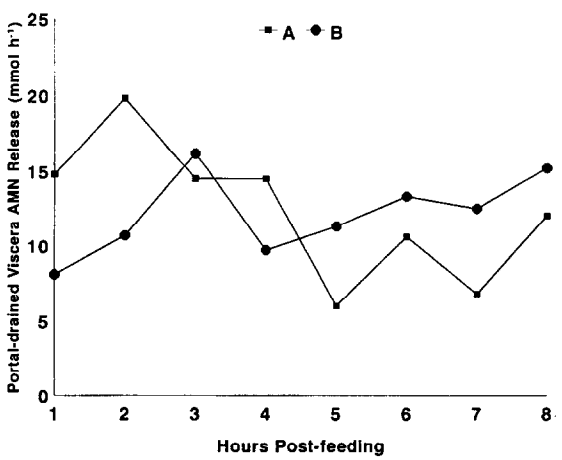

Fig. 7. Portal-drained viscera release of ammonia N (AMN) at different times post-feeding (based on samples collected at listed times, and at 20 min before and after) in wethers consuming 2.0% of BW (DM) of alfalfa or bermudagrass hay. The pooled SE was 2.84 mmol h^{-1} (Experiment 2).

4. Discussion

Legume intake by growing animals is typically greater than that of grasses (Minson, 1990). In our experiment, A consumption may not have differed from grass intake in part because of the relatively high stage of maturity of these wethers. Results of Penning et al. (1991, 1995) suggest that differences between intake of legumes and grasses may decrease with decreasing nutrient requirements. Lower digestible OM intake for B than for RW resulted from less extensive OM digestibility and the numerical difference in DM intake.

Greater idle time for A than for RW or B indicates less energy expended in mastication and associated activities. The interaction between forage treatment and time post-feeding in percentage of time standing was a consequence of a similar interaction in eating time. Perhaps the longer period of time eating following offering RW than B related to numerically greater RW intake, and the difference between RW and A probably involved the more rapid potential rate of ingestion of legume than grass (Penning et al., 1995). Likewise, less time spent ruminating A than grasses reflects differences in NDF concentration and particle disintegration per unit mastication (Chai et al., 1985; Moseley and Dellow, 1985). Although actual feed consumption at different times post-feeding was not estimated, these behavioral results may suggest that even with ad libitum intake most feed was consumed in the first hour offered, and the period of time during which forage became exposed to microbial activity was longest for RW. However, temporal patterns of nutrient absorption are functions of time events other than ingestion pattern alone, including the proportion of soluble nutrients released with mastication, microbial cell and end-production formation, degradation of

insoluble matter, digesta outflow from the rumen and postruminal digestion and absorption.

Portal and hepatic blood flows changed with time post-feeding slightly differently than observed by Mineo et al. (1991) with mature sheep fed once daily a limited quantity of a pelleted alfalfa-based diet (approximately 1.9% BW; DM). In that experiment, blood flows increased from feeding to plateaus 2 to 4 h later, then declined until approximately 8 h after feeding and did not appreciably change thereafter. Factors responsible for this disparity in results are unclear but could involve differences in experimental conditions such as feeding frequency and diet physical form. The interaction between forage treatment and time post-feeding in hepatic arterial blood flow in our experiment appeared largely a result of differences between A and grasses in change with advancing time. Hepatic arterial flow for A was highest among times at 1 h and then declined; whereas, for B and RW flow increased from 1 to 3 h post-feeding.

The greatest difference between treatments in hepatic and splanchnic bed oxygen consumption was at 1 h post-feeding, with uptake substantially greater for A versus grasses. Because of the nonsignificant time effect and absence of a significant interaction between forage treatment and time in PDV oxygen consumption, the temporal pattern in splanchnic bed oxygen consumption was primarily owing to that of the liver. Differences between A and grasses in NDF concentration suggest rapid nutrient release in the rumen and a corresponding peak in microbial fermentative activity early after feeding, which was accompanied by a relatively high proportion of nutrient absorption for A at that time. In relation, hepatic AMN uptake correlated with hepatic oxygen consumption at 1 h post-feeding ($r = 0.89$; $P = 0.02$) but not at other times.

Relatively high hepatic and splanchnic bed energy consumption early after feeding A did not have an obvious effect on energy consumption later in the feeding interval. Thus, the ruminant liver may maintain adequate metabolic machinery necessary for metabolism of nutrients released by the PDV when greatest. However, similar capacity with the associated expenditure of energy may exist for diets with less temporal variation in ruminal availability of nutrients and resultant microbial fermentative activity. Hence, these results imply that differences in the temporal pattern of nutrient absorption among forages consumed ad libitum do not markedly alter splanchnic tissue energy consumption over an entire feeding interval. Although, such differences among forages might occur via other means, such as unique physical characteristics of digesta (e.g. mass) that influence epithelial cell mass and energy consumption (Rompala et al., 1988, 1990). In addition, effects on efficiency of peripheral tissue energy metabolism of the array of nutrients absorbed as altered by forage source are possible as well (Abdul-Razzaq and Bickerstaffe, 1989).

The difference between A and grasses in PDV AAN release could not be explained by digestible OM or DE intakes, as potentially influencing microbial protein synthesis, which is also supported by nonsignificant correlations between these variables. Furthermore, this difference was not expected if it is assumed that PDV amino acid metabolism relative to energy consumption was constant across time post-feeding. Portal-drained viscera release of AMN did not change with advancing time post-feeding for RW and B, and changed only marginally with A. These results in part could relate to AMN arising from amino acids metabolized by PDV tissues, for which the pattern of release may not have coincided with release of absorbed AMN. Furthermore, it did not seem that synchrony of availability to ruminal microbes of AMN and energy from fermentation greatly changed with advancing time after feeding RW and B.

The absence of time effects and interactions between time and forage treatment in UN PDV uptake and hepatic release generally coincide with net fluxes of AMN. However, variation in hepatic UN release was appreciable and values for A and RW could not be totally accounted for by hepatic uptakes of AAN and AMN. Both hepatic uptake of AAN ($r = 0.31$; $P = 0.03$) and of AMN ($r = 0.52$; $P < 0.01$) were significantly related to hepatic UN release. However, correlations and regressions conducted with net fluxes at different times post-feeding did not identify shifts with advancing time in relative importances of uptakes of AAN and AMN as influencers of the quantity of UN released and associated energy use in ureagenesis. With the relatively high stage of maturity of these wethers, associated with submaximal capacity for peripheral protein synthesis, and con-

sumption of forage-based diets that limits peripheral energy availability and thereby restricts peripheral protein accretion relative to diets containing concentrate, an appreciable quantity of AMN taken up by the liver may have originated from peripheral tissues. This would have decreased potential effects of changes with time post-feeding in PDV release of AMN and AAN on hepatic release of UN and uptake of AAN.

Overall, these results suggest that to accurately assess average splanchnic tissue blood flows and oxygen consumption within an 8 h feeding interval with forage diets consumed ad libitum, sampling throughout an entire feeding interval is warranted. Moreover, adequate consideration of the early postprandial period (e.g. first 3 h) seems necessary to adequately evaluate differences among forages such as between legumes and grasses. Sampling scheme might not markedly impact PDV AAN release with grasses but could with legumes. For forages high in cell walls such as grasses used in our experiment, sampling scheme did not appear critical to adequately assess average net fluxes of AMN within an 8 h feeding interval, although for legumes a greater number of samples and ones chosen to represent the entire 8 h feeding interval may be necessary.

4.1. Experiment 2

Based on proportions of time spent eating, almost all feed appeared to be consumed in the first hour after offering, compared with at least small proportions of time spent eating in nearly all hours post-feeding in Experiment 1. Typically, feed restriction decreases eating time (Luginbuhl et al., 1989) relative to ad libitum intake. The difference in eating time between A and B was more marked than that in Experiment 1. Thus, the difference between experiments in time spent eating per unit of DM intake appeared greater for B than for A. However, digestibilities were quite similar within forage source between experiments, suggesting that any resultant effects on subjection to mastication did not sufficiently impact accessibility of fiber to microbial actions to alter digestion extent, given expected longer digesta retention in the rumen with limited intake.

Factors responsible for similar digestibilities between experiments are unclear, since anticipated longer ruminal digesta retention in Experiment 2 with limited intake implies more thorough digestion. Particulate passage rate is relatively slow for tropical grass diets because of factors such as particle entanglement in the digesta mat (Kennedy and Doyle, 1993). This suggests that ruminal digesta retention time and, thus, ruminal fiber digestion, for tropical grasses could be less affected by level of feed intake compared with legumes or temperate grasses. Furthermore, legume digestibility might not be greatly altered by feed intake level because of the relatively high concentration of cell solubles, rapid rate of digestion of potentially digestible cell wall and recalcitrant nature of lignified cell walls (Galyean and Goetsch, 1993).

The temporal pattern of change in portal venous blood flow was similar between experiments in regards to generally lowest flow in the middle portion of the feeding interval, and the magnitude of difference between lowest and highest flows was comparable. Perhaps these findings relate to a moderate degree of feed intake restriction rather than a severe limitation. The particular level of feed intake used was selected to minimize or avert feed consumption other than immediately after feed offering and to decrease consumption time during this period. However, behavioral observations indicated that most feed was consumed in the first hour following feeding in both experiments.

The absence of an effect of time post-feeding on PDV oxygen consumption agrees with results of Experiment 1, depicting change with time in the percentage of oxygen present in blood taken up by the PDV (Experiment 1: 24.7, 29.3, 29.4, 32.3, 31.2, 26.9, 22.7 and 24.7%, SE 1.64; Experiment 2: 20.0, 26.6, 23.9, 28.5, 28.0, 25.7, 24.0 and 24.4% at 1, 2, 3, 4, 5, 6, 7 and 8 h post-feeding, SE 2.10). Portal-drained viscera release of AAN for the two forage sources was quite similar to values in Experiment 1 despite the difference in feed intake. Factors possibly involved in these results include less extensive PDV amino acid metabolism with lower feed intake, and more extensive ruminal or intestinal protein digestion with greater absorption of free amino acids versus peptides. The absence of an effect of time post-feeding on AAN release by the PDV may in part relate to assumed slower ruminal particulate

passage rate or less extensive ingestive mastication with limited feed intake in Experiment 2, possibly compensatory for potential impact of the shorter period of time during which feed was consumed.

The lack of effect of treatment on PDV uptake of UN was in accordance with results of Experiment 1. There appeared greater differences among times in PDV AMN release in this experiment than in the previous one. This may be related to more continual microbial formation of AMN, because of a greater array of different types of forage proteins becoming available and being simultaneously degraded by microbes with ad libitum intake in Experiment 1 versus 2. Overall, it appeared that the sampling scheme required to accurately depict net flux of nutrients across the PDV within an 8 h feeding interval with a slight restriction of forage intake would not markedly differ from that with ad libitum consumption.

4.2. Method of determination

Net fluxes were slightly greater for Method 1 (average of net fluxes determined at different times) than for Method 2 (net fluxes calculated from marker and metabolite concentrations averaged across times). However, magnitudes of these differences and the two interactions noted in Experiment 1 were relatively small. Therefore, little benefit was apparent from calculating net fluxes at different times post-feeding compared with use of average marker and metabolite concentrations, if differences among forage sources in average net fluxes over an 8 h feeding interval are of primary interest.

5. Conclusion

In wethers at a relatively high stage of maturity consuming forage diets ad libitum, A resulted in a different temporal pattern of change with time post-feeding in hepatic and splanchnic bed oxygen consumption in an 8 h feeding interval compared with RW and B. However, it did not appear that high oxygen consumption early after feeding A impacted oxygen consumption at subsequent times in the feeding interval. Thus, differences among forages in efficiency of whole body energy metabolism may not be elicited by effects of the temporal pattern of nutrient absorption as impacted by forage source on splanchnic tissue energy consumption. It seems that samples should be collected throughout an 8 h feeding interval, particularly shortly after feeding, to assess average net flux of nutrients across splanchnic tissues for the interval, with ad libitum consumption or a moderately restricted level of feed intake. There appeared little advantage to estimating average net fluxes over an 8 h feeding interval based on net fluxes at different times or marker and metabolite concentrations averaged over time.

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